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ENVIRONMENTAL BIOTECHNOLOGY

Engineering and characterization of copper and gold sensors in *Escherichia coli* and *Synechococcus* sp. PCC 7002

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Abstract

The anthropogenic release of toxic metals into the environment poses danger to the health of both humans and the local ecosystem. Biosensors for the detection of metals have been developed to improve our ability to monitor these environmental contaminants, yet most of these sensors use heterotrophic bacterial hosts, which require a fixed carbon source and do not typically grow in natural waterways. In this study, we constructed and characterized metal sensors for development of a photoautotrophic biosensor using *Synechococcus* sp. PCC 7002. We characterized gold and copper sensors based on modified MerR transcriptional activators: GolS^{A113T}, with improved gold binding, and GolSCL, containing the metal-binding loop from CueR which binds both gold and copper. The metal-sensing constructs were first optimized and characterized in *Escherichia coli* MG1655. The addition of a strong ribosome binding site to the optical reporter protein increased translation of the fluorescent reporter, and expression of *golS*^{A113T} from the *rbc* promoter of *Synechococcus* sp. PCC 7002 improved the response to gold in MG1655. In rich medium, the GolS^{A113T}-based *E. coli* sensor detected gold at concentrations as low as 100 nM, while the GolSCL-based *E. coli* sensor detected gold and copper at sensitivities of 100 nM and 10 μM, respectively. Both *E. coli* sensors responded to gold and copper yet showed no detectable response to other metals. Abiotic factors, such as medium complexity, were found to influence the response of the *E. coli* sensors, with minimal medium resulting in higher sensitivities of detection. Expression of the GolS^{A113T}- and GolSCL-based sensor constructs in the cyanobacterium *Synechococcus* sp. PCC 7002 resulted in photoautotrophic gold sensors, but these biosensors failed to produce a significant response to copper. Moreover, the fluorescence response of the cyanobacterial sensors to gold was significantly reduced compared to that of analogous *E. coli* sensors. While this effort demonstrates feasibility for the development of photoautotrophic biosensors, additional efforts to optimize sensor performance will be required.

Keywords Gold biosensor · Copper biosensor · Metal biosensor · Cyanobacterial biosensor · *Synechococcus* sp. PCC 7002 · GolS

Introduction

The accidental release of heavy metals into the environment, such as in the Gold King Mine spill of 2015, poses risks to human, animal, and ecosystem health (Rodriguez-Freire et al. 2016). The detection of heavy metal contamination across such large geographical areas is challenging using conventional sampling and laboratory analysis. Biological sensors may facilitate the detection of toxic heavy metals through portable field devices for in situ measurements.

MerR family proteins bind transition metals and activate transcriptional responses upon metal binding (Brown et al. 2003). Many MerR family proteins, such as MerR, ZntR, CueR, CadR, and PbrR, bind and respond to several different metals, yet mutation of the metal-binding loop may enable high specificity for a single metal target (Brocklehurst et al.

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1999; Brown et al. 2003; Ibanez et al. 2013; Khan et al. 2002). As metal ion binding directly translates into transcriptional activation, MerR family proteins have been used to develop genetically modified sensors for mercury, zinc, copper, cadmium, lead, and gold in bacterial hosts of *Escherichia coli*, *Salmonella enterica*, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas fluorescens*, or *Pseudomonas putida* (Bereza-Malcolm et al. 2016; Bondarenko et al. 2008; Cerminati et al. 2011, 2015; Charrier et al. 2011; Corbisier et al. 1999; Hakkila et al. 2004; Hansen and Sørensen 2000; Hurdebise et al. 2015; Ivask et al. 2009; Ivask et al. 2002; Kang et al. 2018; Li et al. 2014; Priyadarshi et al. 2012; Riether et al. 2001; Tseng et al. 2014; Virta et al. 1995; Zammit et al. 2013). These engineered biosensors produce optical responses (e.g., fluorescent proteins or bioluminescence) at metal sensitivities in the parts per billion (ppb) or even parts per trillion (ppt) range with varying metal specificity (Ivask et al. 2009; Zammit et al. 2013). Targeted mutation of the metal-binding loop of MerR family proteins has improved metal specificity, such as the A113T mutation of GolS, which reduced copper activation of GolS to improve gold specificity (Ibanez et al. 2013). While these MerR-based sensors successfully demonstrate metal detection under laboratory conditions, heterotrophic bacterial sensors will be challenging to implement in the field due to their temperature and nutrient requirements as well as public health concerns associated with using pathogenic hosts such as *S. enterica* or *S. aureus*.

Photoautotrophic organisms, like cyanobacteria, are ideal hosts for environmental biological sensors. They do not require a fixed carbon source that may promote contamination, and they can persist with minimal nutrients supplied in natural aqueous environments. The cyanobacterium *Synechococcus* sp. PCC 7002 is a robust host for metal sensing; it has high salt and high light tolerance along with characterized tools for genetic modification (Ludwig and Bryant 2012; Markley et al. 2014; Ruffing et al. 2016). In this study, we engineered *E. coli* and *Synechococcus* sp. PCC 7002 as biosensors for the detection of gold and copper by introducing the GolS^{A113T} transcriptional activator derived from *S. enterica* and GolSCL, in which the metal-binding loop of GolS was replaced with the metal-binding loop from CueR (Cerminati et al. 2011; Checa et al. 2007). These bacterial sensors produce an optical fluorescence response to the addition of either gold or copper (Fig. 1). Both the promoter and ribosome binding site (RBS) for *golS*^{A113T} transcription and translation were modified to optimize performance of the gold sensor. The *E. coli* sensors were characterized to determine sensitivity and specificity for gold and copper sensing, and other environmental factors, such as metal oxidation state and matrix effects, were tested. Lastly, the gold and copper sensors were expressed in *Synechococcus* sp. PCC 7002 to demonstrate functionality for environmental metal sensing in this photoautotrophic host.

Materials and methods

Materials

Chemicals used in this study were manufactured by Genlantis (kanamycin monosulfate), Invitrogen (SYBRsafe), ACROS Organics ($Na_2MoO_4{\cdot}2H_2O$ and NH4Cl), Alfa Aesar (agar and gold sodium thiosulfate), MP Biomedicals ($CuSO_4{\cdot}2H_2O$, $CoCl{\cdot}6H_2O$, and thiamine HCl), Sigma Aldrich ($CuCl_2{\cdot}2H_2O$, $FeCl_3{\cdot}6H_2O$, KH_2PO_4, $MgCl_2{\cdot}7H_2O$, $MnCl_2{\cdot}2H_2O$, $Na_2EDTA{\cdot}2H_2O$, vitamin B_{12}, and $ZnCl_2$), Fisher Bioreagents (Luria Broth, NaCl, and $CsCl_2$), and Fisher Scientific (all other chemicals). Plasmid isolation, PCR purification, and gel extraction kits used for strain construction were purchased from Qiagen. Primers and gblocks were purchased from Integrated DNA Technologies (IDT). All enzymes used for strain construction and verification (Q5 and Taq DNA polymerases, quick DNA ligase, Gibson Assembly master mix, and restriction enzymes) were obtained from New England Biolabs.

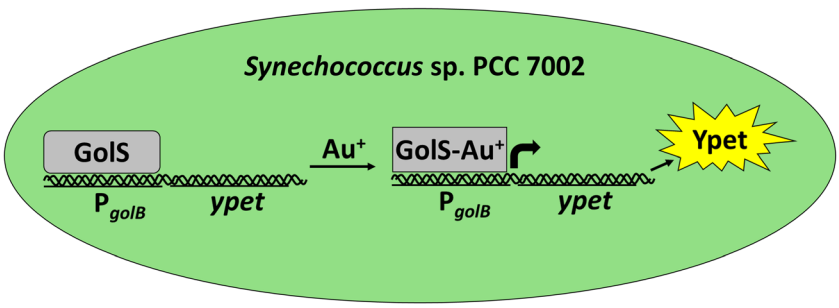

Fig. 1 Schematic of a GolS-based biosensor in *Synechococcus* sp. PCC 7002. In the absence of gold, GolS functions to repress transcription of genes downstream of P_{golB}. Upon binding to gold, GolS undergoes a conformational change leading to transcription of downstream genes. Our system is engineered for transcriptional control of *ypet* by GolS^{A113T}, allowing for production of a detectable fluorescent signal upon exposure to gold

Strain construction

All strains used and constructed in this study are listed in Table 1. Plasmids expressing *ypet* from three different *Synechococcus* sp. PCC 7002 promoters ($pSBP_{1173}$*ypet*, $pSBP_{rbc}$*ypet*, and $pSBP_{2579}$*ypet*) were used to measure promoter strength; these were previously constructed (Ruffing et al. 2016). Gold sensor plasmids, containing $golS^{A113T}$ and *ypet* driven by the *golB* promoter, were constructed. The $golS^{A113T}$ gene (codon optimized for expression in *Synechococcus* sp. PCC 7002), *psbA* terminator, and the *golB* promoter ($golS^{A113T}$-T_{psbA}-P_{golB}) were synthesized as a gblock by IDT (sequence in Online Resource 1). The $golS^{A113T}$-T_{psbA}-P_{golB} gblock was integrated into previously constructed plasmid backbones (Ruffing et al. 2016). The plasmid backbones were amplified from $pSBP_{1173}$*ypet* and $pSBP_{rbc}$*ypet* using primers listed in Table S1 (Online Resource 1) (Ruffing et al. 2016). Primers were used to insert overhangs on the $golS^{A113T}$-T_{psbA}-P_{golB} gblock for insertion via Gibson Assembly to produce the gold sensor constructs pGS01 and pGS02, which drive $golS^{A113T}$ expression with P_{1179} and P_{rbc}, respectively. A strong ribosome binding site (RBS) from locus SynPCC7002_A2579 was inserted upstream of *ypet* in both pGS01 and pGS02 to construct pGS03 and pGS04. To insert the strong SynPCC7002_A2579 promoter upstream of $golS^{A113T}$, primers were designed with *Kpn*I and *Nde*I restriction sites for amplification of P_{2579} and the gold sensor backbone of pGS04 with removal of P_{rbc}. Digestion and ligation of the two purified fragments yielded pGS05. To generate a copper-responsive GolS sensor (GolSCL), amino acid residues 111–120 (CCTGDALPDC), constituting the metal-binding loop of GolS, were replaced with the corresponding metal-binding loop residues of CueR (SCPGDDSADC) (Ibanez et al. 2013). To do this, an *E. coli* codon-optimized *golSCL* was synthesized by IDT (Online Resource 1). Synthesized *golSCL* was then PCR amplified using primers with 20 base pair overhangs homologous to the 5′ and 3′ ends of pGS02 surrounding $golS^{A113T}$. The PCR product was purified and inserted into pGS02 via Gibson Assembly, replacing $golS^{A113T}$. Primers used for amplification of pGS02 with removal of $golS^{A113T}$ can be found in Table S1. The resulting plasmid, pCS01, expresses *golSCL* from the *rbc* promoter with P_{golB} upstream of *ypet.* All constructs were verified by colony PCR screening and Sanger sequencing (Eurofins Genomics). All plasmid maps (Fig. S1) and sequences can be found in Online Resource 1.

The gold sensor and copper sensor plasmids were transformed into *E. coli* MG1655 competent cells using heat shock (Sambrook et al. 2001). After selection on LB agar plates containing 50 μg/mL of kanamycin monosulfate, successful transformation was confirmed with colony PCR screening. For transformation of *Synechococcus* sp. PCC 7002, the sensor plasmids were linearized by *Spe*I digestion and purified using the QIAquick PCR purification kit (Qiagen). The linearized plasmids were transformed as described previously (Ruffing 2014; Stevens and Porter 1980). Following selection on medium A+ agar plates containing 50 μg/mL of

Table 1 Biological strains used for gold and copper sensing

Strain	Description	Source
Escherichia coli DH5α	Wild-type *E. coli* strain used for molecular cloning	NEB
Escherichia coli MG1655	*E. coli* MG1655 strain used for constructing gold sensors	Coli Stock Center
MG1655/$pSBP_{1173}$Ypet	*E. coli* MG1655 containing a plasmid with the operon P_{1173}-*ypet*	This study
MG1655/$pSBP_{rbc}$Ypet	*E. coli* MG1655 containing a plasmid with the operon P_{rbc}-*ypet*	This study
MG1655/$pSBP_{2579}$Ypet	*E. coli* MG1655 containing a plasmid with the operon P_{2579}-*ypet*	This study
MG1655/pGS01	*E. coli* MG1655 containing a plasmid with the operon P_{1173}-$golS^{A113T}$-P_{golB}-*ypet*	This study
MG1655/pGS02	*E. coli* MG1655 containing a plasmid with the operon P_{rbc}-$golS^{A113T}$-P_{golB}-*ypet*	This study
MG1655/pGS03	*E. coli* MG1655 containing a plasmid with the operon P_{1173}-$golS^{A113T}$-P_{golB}-RBS_{2579}-*ypet*	This study
MG1655/pGS04	*E. coli* MG1655 containing a plasmid with the operon P_{rbc}-$golS^{A113T}$-P_{golB}-RBS_{2579}-*ypet*	This study
MG1655/pGS05	*E. coli* MG1655 containing a plasmid with the operon P_{2579}-$golS^{A113T}$-P_{golB}-RBS_{2579}-*ypet*	This study
MG1655/pCS01	*E. coli* MG1655 containing a plasmid with the operon P_{rbc}-*golSCL*-P_{golB}-RBS_{2579}-*ypet*	This study
Synechococcus sp. PCC 7002	Wild-type marine cyanobacterial strain ATCC	ATCC
SynGS01	*Synechococcus* sp. PCC 7002 with P_{1173}-$golS^{A113T}$-P_{golB}-RBS_{2579}-*ypet* from pGS03 genome integrated at putative NS2	This study
SynGS02	*Synechococcus* sp. PCC 7002 with P_{rbc}-$golS^{A113T}$-P_{golB}-RBS_{2579}-*ypet* from pGS04 genome integrated at putative NS2	This study
SynGS03	*Synechococcus* sp. PCC 7002 with P_{2579}-$golS^{A113T}$-P_{golB}-RBS_{2579}-*ypet* from pGS05 genome integrated at putative NS2	This study
SynCS01	*Synechococcus* sp. PCC 7002 with P_{rbc}-*golSCL*-P_{golB}-RBS_{2579}-*ypet* from pCS01 genome integrated at putative NS2	This study

kanamycin monosulfate, single colonies were streaked on selection plates. After three rounds of streaking and selection to segregate homologous genome copies, genomic integration at neutral site 2 (NS2) and segregation were confirmed using colony PCR with NS2_5reF and NS2_3reR primers (Ruffing et al. 2016).

Cultivation conditions

E. coli strains MG1655, MG1655/pSBP$_{1173}$*ypet*, MG1655/pSBP$_{rbc}$*ypet*, MG1655/pSBP$_{2579}$*ypet*, MG1655/pGS01, MG1655/pGS03, MG1655/pGS04, MG1655/pGS05, and MG1655/pCS01 were inoculated in 4 mL of LB broth with 50 μg/mL of kanamycin monosulfate for modified strains in 16-mm borosilicate glass test tubes with vented caps and cultured at 37 °C and 200 rpm overnight in an Innova 42R shaking incubator. Each culture was then diluted into a series of 125-mL baffled flasks with a final volume of 25 mL at $OD_{600} = 0.1$ using either LB or supplemented M9 minimal medium (SM9) containing 0.4% glucose, 1 μg/mL thiamine HCl, and 0.2% casamino acids (Stoyanov and Brown 2003). Gold trichloride stock solution (1000 ppm) was added to a final concentration of 10 μM for preliminary gold sensor testing, and sterile stocks of 5 mM $CuCl_2$ were added to a final concentration of 100 μM for preliminary copper sensor testing. Cultures were grown in the aforementioned conditions, and cell growth and Ypet fluorescence were monitored over time (see "Spectroscopy measurements").

Synechococcus sp. PCC 7002, SynGS01, SynGS02, SynGS03, and SynCS01 sensor strains were inoculated from medium A+ agar plates into 4 mL of medium A+ (Stevens et al. 1973) with 50 μg/mL of kanamycin monosulfate for sensor strains in 16-mm borosilicate glass test tubes with vented caps. The inoculums were placed in an Innova 42R shaking incubator at 30 °C with agitation at 150 rpm and 60 μmol photons m^{-2} s^{-1} illumination from alternating cool white and plant fluorescent lights. After approximately 5 to 6 days of growth, the inoculums were transferred into 25 mL of medium A+ (with antibiotics as needed) in a 125-mL baffled Erlenmeyer flask with vented cap. The gold trichloride stock solution (1000 ppm, Fisher Scientific) was added to a final concentration of 1 μM, and a sterile stock of 5 mM $CuCl_2$ was added to a final concentration 10 μM for sensor testing. Cultures were grown in the aforementioned conditions, and cell growth and Ypet fluorescence were monitored over time (see "Spectroscopy measurements").

Sensitivity and specificity testing of biosensors

For sensitivity testing, cultures of MG1655/pGS04 or MG1655/pCS01 were treated with increasing $AuCl_3$ concentrations at 1 nM, 10 nM, 100 nM, 1 μM, 10 μM, and 100 μM or increasing $CuCl_2$ concentrations at 1 nM, 10 nM, 100 nM, 1 μM, 10 μM, 100 μM, and 1 mM. For metal specificity testing, 1000 ppm of $AuCl_3$ and 50 mM of $CuCl_2$, $MnCl_2$, $CaCl_2$, $ZnCl_2$, $MgCl_2$, $CoCl_2$, $AgNO_3$, and $FeCl_3$ were used as metal stock solutions. Cultures each containing 25 mL of MG1655/pGS04 or MG1655/pCS01 ($OD_{600} = 0.1$) in 125-mL baffled Erlenmeyer flasks were treated with 10 μM of each metal. For both sensitivity and specificity testing, 20 μL of 0.01 mM HCl was added to one of the cultures and served as the control. As specified, the growth medium was either LB or SM9. Samples were diluted 20-fold before taking fluorescence and absorbance measurements (see "Spectroscopy measurements").

Spectroscopy measurements

For the *E. coli* cultures, fluorescence and absorbance measurements were acquired using a BioTek Synergy H4 microplate reader with 200 μL of culture in each well of a Costar black bottom or clear-bottom 96-well plate for fluorescence or absorbance, respectively. All fluorescence measurements used a gain of 100 and read height of 5 mm. Ypet fluorescence was measured with 500/20 nm excitation and 530/20 nm emission detection. For *E. coli* sensor cultures, the samples were diluted so that the fluorescence reading was within the detection limits of the microplate reader. For absorbance measurements, *E. coli* cultures were diluted identical to the fluorescence measurements for the gold and copper sensor tests (MG1655/pGS01, MG1655/pGS03, MG1655/pGS04, MG1655/pGS05, and MG1655/pCS01), and absorbance was measured at 600 nm for all *E. coli* cultures. For *Synechococcus* sp. PCC 7002 and cyanobacterial sensor cultures, absorbance was measured at 730 nm with cultures diluted such that the absorbance measurement remained under $OD_{730} = 0.5$. Fluorescence measurements were taken with undiluted samples due to the low fluorescence emission from the cyanobacterial cultures. Relative OD-normalized fluorescence values were calculated using the following:

$$\frac{\left(F/_{\mathrm{OD}}\right)_{+\mathrm{metal}}}{\left(F/_{\mathrm{OD}}\right)_{\mathrm{ref}}} \quad (1)$$

where F is the raw fluorescence read, OD is the absorbance reading at 600 nm (*E. coli*) or 730 nm (cyanobacteria), metal is either gold or copper, and ref is the control without gold, either the wild-type strain or the sensor strain without metal, as specified in the figure captions.

For the cyanobacterial gold sensors, Ypet fluorescence was measured using flow cytometry to improve sensitivity of the response relative to bulk fluorescence measurements using the microplate reader. The cyanobacterial cultures were diluted 10 to 1000× in PBS buffer, such that the rate of measurement in the flow cytometer was less than 500 events/s. Diluted

samples were analyzed using an Accuri C6 flow cytometer with a flowrate of 35 μL/min and a sampling rate of 10,000 events within the gated cell population. Ypet fluorescence was measured using the FL1-A filter (Em 533/30 nm), and chlorophyll-*a* fluorescence was measured using the FL3-A filter (Em > 670 nm).

RNA isolation and RT-qPCR

MG1655/pGS01, MG1655/pGS03, MG1655/pGS04, and MG1655/pGS05 were grown with 10 μM gold, as described previously. After 12 h of incubation, Ypet expression was confirmed by fluorescence spectroscopy (see "Spectroscopy measurements"), and 10 mL of each culture was centrifuged for 4 min at 3900×*g* and 4 °C. The supernatant was decanted, and cell pellets were flash-frozen in liquid nitrogen and stored at − 80 °C. Frozen pellets were thawed on ice, and RNA was extracted using a hot acid phenol-chloroform extraction method, described previously for *Synechococcus elongatus* PCC 7942 (Ruffing 2013). After adding 1 volume of absolute ethanol to the extracted RNA, the mixture was further purified and concentrated using a QuickRNA Mini-Prep kit (Zymo Research). DNA was removed from the extracted RNA using a TURBO DNA-free kit (Ambion). RNA was quantified using a Quant-iT RiboGreen RNA assay kit (Invitrogen), and quality was determined by running an RNA Nano6000 chip on an Agilent Bioanalyzer 2100, following the manufacturer's instructions. RNA (5 μg) was converted into cDNA using SuperScript III First Strand Synthesis system (Invitrogen), and each sample included a no reverse transcriptase (RT) control reaction to confirm DNA removal from the RNA sample. Transcript abundance was quantified by mixing 1 μL of 10× diluted cDNA, the primers listed in Table S1 (Online Resource 1) at a final concentration of 200 nM, and Fast SYBR Green Master Mix (Applied Biosystems) and running relative qPCR on an Applied Biosystems 7500 Real Time PCR system. A standard melt curve was run after each qPCR to confirm amplification of only a single product. Three technical replicates and three biological replicates were included for each RT-qPCR measurement, along with a no RT control for each RNA sample. The reference gene for *E. coli* was *gapA* (Viveiros et al. 2007), and the $\Delta\Delta C_T$ method was used for relative quantification (Schmittgen and Livak 2008).

Protein isolation and Western blot

MG1655/pGS01 and MG1655/pGS03 were grown with 10 μM gold, as described previously. After 12 h of incubation, Ypet expression was confirmed by fluorescence spectroscopy (see "Spectroscopy measurements"), and 5 mL of each culture was centrifuged at 3900×*g* and 4 °C for 10 min for protein extraction. Cell pellets were washed once with 5 mL of sterile ultrapure water and centrifuged a second time. Laemmli buffer (500 μL) (Karlsson et al. 1994) was added to resuspend each cell pellet. The resuspended cells were heated in boiling water for 5 min and then centrifuged at 14,000×*g* for 5 min to remove cell debris. Protein samples were analyzed using SDS-PAGE (4–20% Mini-PROTEAN TGX gel, Bio-Rad) with SimplyBlue SafeStain (Invitrogen). The SDS-PAGE gel was imaged on a Bio-Rad Chemi-Doc XRS+ system, and protein bands from four different molecular weights were used to quantify sample loading. For the Western blot, the samples were loaded based on the SDS-PAGE gel quantification for normalization. The proteins were transferred to a nitrocellulose membrane (Protran Western blotting membranes, Amersham) using the Bio-Rad Mini Trans-Blot module and the manufacturer's instructions. Anti-GFP polyclonal antibody (ThermoFisher A-11122, at 1:5000 dilution) was used as the primary antibody to detect Ypet, and goat anti-rabbit-HRP (ThermoFisher 31462, at 1:10,000 dilution) was used as the secondary antibody. Precision Plus Protein WesternC standards (Bio-Rad) were used as the ladder, along with the provided Precision Protein StrepTactin-HRP conjugate (Bio-Rad, at 1:5000 dilution). SuperSignal West Pico Chemiluminescent Substrate (1 mL, ThermoScientific) was added for imaging with 10 s exposure time. Three biological replicates were performed for each condition.

Accession numbers for synthetic sequences

The synthetic gene sequences for $golS^{A113T}$ and *golSCL* were submitted to GenBank: accession numbers MH379092 and MH379091. The sequences can also be found in Online Resource 1.

Results

Optimization of gold and copper sensors in *E. coli*

The GolS-based sensors developed in this study include three main components: the metal-responsive transcriptional activator ($GolS^{A113T}$ or GolSCL), the GolS-regulated *golB* promoter (P_{golB}), and the optical reporter (Ypet). To optimize GolS-based metal sensors, we first tested the influence of the ribosome binding site (RBS) on gold sensor performance. The native RBS from the *golB* promoter of *S. enterica* and the RBS from a strong promoter in *Synechococcus* sp. PCC 7002 (SynPCC7002_A2579) were inserted upstream of *ypet* in the gold sensor construct and transformed into the *E. coli* host to yield MG1655/pGS01 and MG1655/pGS03, respectively (Table 1). The strong A2579 RBS led to more than a twofold increase in Ypet fluorescence emission relative to the native *golB* RBS with the addition of 10 μM of $AuCl_3$ (Fig. 2a). However, background Ypet fluorescence emission also increased in MG1655/pGS03, such that the fold change

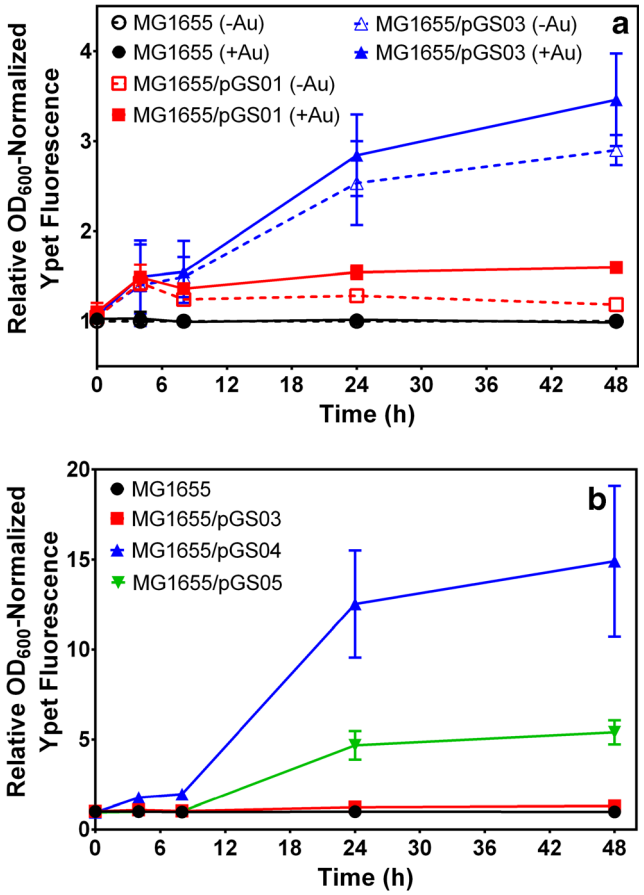

Fig. 2 Optimization of gold sensors in *E. coli* MG1655. **a** Optimization of RBS strength for *ypet* by measuring the relative OD_{600}-normalized Ypet fluorescence over time in LB medium for MG1655, MG1655/pGS01, and MG1655/pGS03 with and without the addition of 10 μM $AuCl_3$. The OD_{600}-normalized Ypet fluorescence values for MG1655 with 10 μM HCl at each timepoint were used as the reference in Eq. 1. **b** Optimization of $golS^{A113T}$ promoter strength by measuring relative OD_{600}-normalized Ypet fluorescence over time in LB medium for MG1655, MG1655/pGS03, MG1655/pGS04, and MG1655/pGS05 with and without the addition of 10 μM $AuCl_3$. The OD_{600}-normalized Ypet fluorescence values for each strain without the addition of $AuCl_3$ at each timepoint were used as the reference in Eq. 1. Data are averages of at least three biological replicates, with error bars indicating the standard deviation

in response to $AuCl_3$ was similar to the MG1655/pGS01 sensor. Transcript and protein abundances were measured using RT-qPCR and a Western blot, confirming that Ypet protein levels were elevated in MG1655/pGS03 relative to MG1655/pGS01 and that transcript levels did not change significantly (Online Resource 1, Fig. S2).

Next, we varied expression of the gold-responsive transcriptional activator, $GolS^{A113T}$. The *Synechococcus* sp. PCC 7002 promoters A1173, *rbc*, and A2579 were previously shown to produce low, moderate, and high expression of Ypet in *Synechococcus* sp. PCC 7002 (Ruffing et al. 2016). These constructs, $pSBP_{1173}$Ypet, $pSBP_{rbc}$Ypet, and $pSBP_{2579}$Ypet, were transformed into *E. coli* MG1655 to measure expression. Ypet fluorescence emission was monitored over a 48-h time period in MG1655/$pSBP_{1173}$Ypet, MG1655/$pSBP_{rbc}$Ypet, and MG1655/$pSBP_{2579}$Ypet (Fig. S3). Similar levels of Ypet fluorescence were observed in MG1655/$pSBP_{rbc}$Ypet and MG1655/$pSBP_{2579}$Ypet, while MG1655/$pSBP_{1173}$Ypet produced more than fivefold lower Ypet fluorescence at 24 h. The A1173, *rbc*, and A2579 promoters were then placed upstream of $golS^{A113T}$ to produce the gold sensor constructs pGS03, pGS04, and pGS05, which include the A2579 RBS for enhanced Ypet translation (Table 1 and Fig. S1). Performance of these gold sensor constructs was evaluated in *E. coli* MG1655 with and without the addition of 10 μM of $AuCl_3$ (Fig. 2b). MG1655/pGS03 showed a slight increase in OD_{600}-normalized Ypet fluorescence at 24 and 48 h after gold addition, 1.24- and 1.30-fold changes relative to without $AuCl_3$. However, a one-tailed Student's *t* test suggests that these fold changes in OD_{600}-normalized Ypet fluorescence are not statistically significant, with *p* values of 0.0835 and 0.0887 for the 24- and 48-h timepoints. The other two gold sensors, MG1655/pGS04 and MG1655/pGS05, showed a similar increase in Ypet fluorescence up to about 8 h, but MG1655/pGS04 produced more than 2.5-fold higher relative OD_{600}-normalized fluorescence compared to MG1655/pGS05 at 24 and 48 h. While the *rbc* and A2579 promoters had similar promoter strengths in MG1655 (Fig. S3), expression of $golS^{A113T}$ from the *rbc* promoter resulted in improved performance of the gold sensor relative to $golS^{A113T}$ expression from the A2579 promoter (Fig. 2b). By directly measuring transcript levels with RT-qPCR, we determined that $golS^{A113T}$ and *ypet* transcripts correlated with Ypet fluorescence measurements, confirming that increased $golS^{A113T}$ transcription led to the improvement in sensor response for MG1655/pGS04 (Table S2, Online Resource 1). Therefore, MG1655/pGS04 was selected for further characterization.

Previous studies showed that the amino acid residues of the GolS metal-binding loop provide it with unique specificity for gold (Ibanez et al. 2013, 2015). Interestingly, replacing the metal-binding loop of GolS with that of CueR allowed GolS to respond to both gold and copper. Thus, we sought to develop a dual gold and copper sensor by replacing residues 111–120 (CCTGDALPDC) of GolS with the corresponding residues of CueR (SCPGDDSADC). This dual gold and copper sensor (*golSCL*) was inserted into pGS04, replacing $golS^{A113T}$ to yield pCS01 (Fig. S1, Online Resource 1). We tested the functionality of MG1655/pCS01 by monitoring Ypet fluorescence and OD_{600} values over time. Slight increases in OD_{600}-normalized Ypet fluorescence over background were observed with addition of 10 μM $AuCl_3$ or 100 μM $CuCl_2$ as early as 4 h post metal addition, with the largest increases observed at 24 and 48 h (Fig. 3). At these initial concentrations tested, both gold

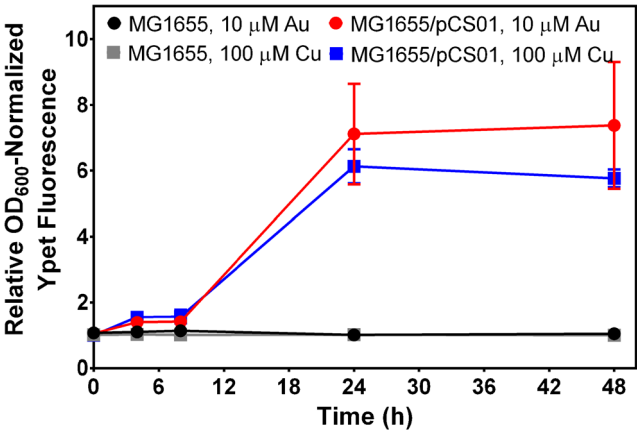

Fig. 3 Evaluation of the GolSCL-based *E. coli* sensor MG1655/pCS01 compared to wild-type MG1655 in response to 10 μM $AuCl_3$ or 100 μM $CuCl_2$ addition in LB medium. The OD_{600}-normalized Ypet fluorescence values for each strain without the addition of metal at each timepoint were used as the reference in Eq. 1. Data are averages of at least three biological replicates, with error bars indicating the standard deviation

and copper led to similar levels of maximal activation: six- to sevenfold over background. Thus, GolSCL can function as a sensor for both gold and copper.

Characterization of *E. coli* gold and copper sensors

We next sought to fully characterize the metal sensitivity and specificity of both the $GolS^{A113T}$- and GolSCL-based sensors. To determine gold sensitivity of $GolS^{A113T}$, MG1655/pGS04 cultures at a starting OD_{600} of 0.1 were exposed to increasing concentrations of $AuCl_3$ in LB medium: 1 nM, 10 nM, 100 nM, 1 μM, 10 μM, and 100 μM. $GolS^{A113T}$-based activation of Ypet expression was monitored over 24 h and plotted as the relative OD_{600}-normalized fluorescence at 530 nm (Fig. 4a). No significant fluorescence response was detected at gold concentrations up to 10 nM. MG1655/pGS04 showed slight activation at 100 nM of $AuCl_3$, yet the largest increase in relative OD_{600}-normalized Ypet fluorescence was observed at 1 μM of $AuCl_3$, with reduced responses at 10 μM and 100 μM of $AuCl_3$. Addition of 100 μM $AuCl_3$ resulted in a longer lag phase as shown in Fig. S4 (Online Resource 1), which is consistent with the delayed increase in Ypet fluorescence (Fig. 4a). In LB medium, exposure of MG1655/pGS04 cultures to $CuCl_2$ concentrations of 1 nM, 10 nM, 100 nM, and 1 μM elicited no detectable increase in OD_{600}-normalized Ypet fluorescence, while $CuCl_2$ concentrations of 10 μM, 100 μM, and 1 mM resulted in very slight (9 to 17%) increases in OD_{600}-normalized Ypet fluorescence (Fig. 4b). The sensitivity of the GolSCL-based sensor, MG1655/pCS01, was determined using the same $AuCl_3$ and $CuCl_2$ concentrations. Overall, MG1655/pCS01 showed lower relative OD_{600}-normalized Ypet fluorescence compared to MG1655/pGS04 for gold; however, MG1655/

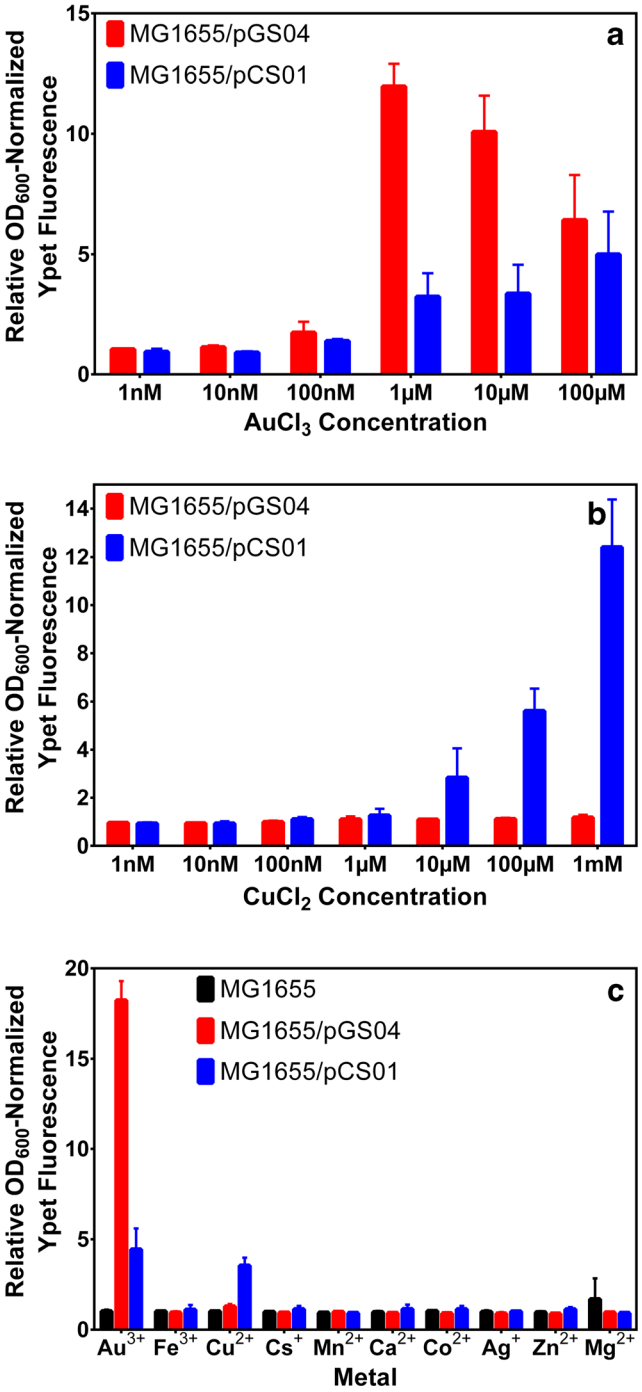

Fig. 4 Characterization of gold and copper sensors. **a**, **b** Sensitivity characterization of the *E. coli* sensors MG1655/pGS04 and MG1655/pCS01 in LB medium with varying concentrations of $AuCl_3$ (**a**) and $CuCl_2$ (**b**). The OD_{600}-normalized Ypet fluorescence values for each strain without the addition of metal were used as the reference in Eq. 1. Measurements were taken 24 h after addition of metal to cultures at a starting cell density of $OD_{600} = 0.1$. **c** Specificity characterization of the *E. coli* sensors MG1655/pGS04 and MG1655/pCS01 along with wild-type MG1655 in LB medium 24 h after addition of 10 μM of each metal. The OD_{600}-normalized Ypet fluorescence values for each strain without the addition of metal were used as the reference in Eq. 1. Data are averages of at least three biological replicates, with error bars indicating the standard deviation

pCS01 displayed a concentration-dependent response to $AuCl_3$, with increasing relative OD_{600}-normalized Ypet fluorescence from 100 nM to 100 μM of $AuCl_3$ (Fig. 4a). MG1655/pCS01 exposed to $CuCl_2$ concentrations up to 1 μM did not elicit a detectable increase in Ypet fluorescence above background levels. Meanwhile, $CuCl_2$ concentrations of 10 μM, 100 μM, and 1 mM each led to increasing levels of activation for MG1655/pCS01 (Fig. 4b).

To determine the metal activation specificity of both $GolS^{A113T}$ and GolSCL, the *E. coli* sensors were exposed to a broad spectrum of metals. MG1655/pGS04 and MG1655/pCS01 cultures in LB medium were treated with 10 μM of $AuCl_3$, $FeCl_3$, $CuCl_2$, $CsCl_2$, $MnCl_2$, $CaCl_2$, $CoCl_2$, $AgNO_3$, $ZnCl_2$, and $MgCl_2$. Changes in Ypet fluorescence and culture growth were monitored over 24 h (Fig. 4c). At this metal concentration (10 μM), no toxicity was observed for any metal based on culture growth measurements (Fig. S5, Online Resource 1). Both MG1655/pGS04 and MG1655/pCS01 showed an increase in OD_{600}-normalized Ypet fluorescence in response to both gold and copper, with MG1655/pGS04 having a 14-fold greater response for gold relative to copper and MG1655/pCS01 showing only a 26% increase in response to gold relative to copper. In contrast, no other metal produced a fluorescence response above background detection levels.

Effect of abiotic factors on *E. coli* gold and copper sensors

Metal ions interact and complex with other chemical constituents present in the testing medium, altering the availability of free metal ion both extracellularly and intracellularly. The above characterizations of MG1655/pGS04 and MG1655/pCS01 were performed in Luria broth (LB), a rich medium (Sridhar and Steele-Mortimer 2016). We sought to determine to what extent the matrix of the *E. coli* sensors affects activation of $GolS^{A113T}$ and GolSCL by comparing the sensitivities of metal activation in LB to sensitivities in supplemented M9 minimal medium (SM9) (Checa et al. 2007). In SM9 medium, MG1655/pGS04 showed a fourfold increase in OD_{600}-normalized Ypet fluorescence 24 h after addition of 100 nM of $AuCl_3$ while only a 1.7-fold increase was observed in LB with the same $AuCl_3$ concentration (Figs. 5a and 4a). Furthermore, MG1655/pGS04 had a 1.6-fold increase in OD_{600}-normalized Ypet fluorescence 24 h after addition of 10 nM of $AuCl_3$ in SM9 medium compared to no significant response in LB. Growth inhibition of MG1655/pGS04 occurred at a lower gold concentration in SM9 medium (10 μM $AuCl_3$) relative to LB medium (100 μM $AuCl_3$) (supplemental Figs. S6 and S4, Online Resource 1). In contrast, MG1655/pCS01 showed little difference in sensitivity to $AuCl_3$ in SM9 medium compared to LB medium, yet the relative OD_{600}-normalized Ypet fluorescence was lower with SM9 medium (2.65 ± 0.38 at 10 μM $AuCl_3$) compared to LB medium (4.99 ± 1.77 at 10 μM $AuCl_3$) (Figs. 5a and 4a). MG1655/pCS01 was more sensitive to low levels of $CuCl_2$ in SM9 medium, with activation observed at 1 μM $CuCl_2$ (Fig. 5a). Similar to the response to gold, copper activation yielded a lower relative OD_{600}-normalized Ypet fluorescence response in SM9 medium compared to LB medium (6.19 ± 2.22 vs 12.4 ± 1.98 at 1 mM $CuCl_2$). The temporal response to most $AuCl_3$ and $CuCl_2$ concentrations showed increasing relative OD_{600}-normalized Ypet fluorescence in SM9 medium with the exception of MG1655/pCS01 exposed to 1 mM $CuCl_2$, which displayed a reduced response after 24 h (Fig. S7, Online Resource 1). Collectively, these results indicate that the *E. coli* gold and copper sensors can function in both chemically complex and

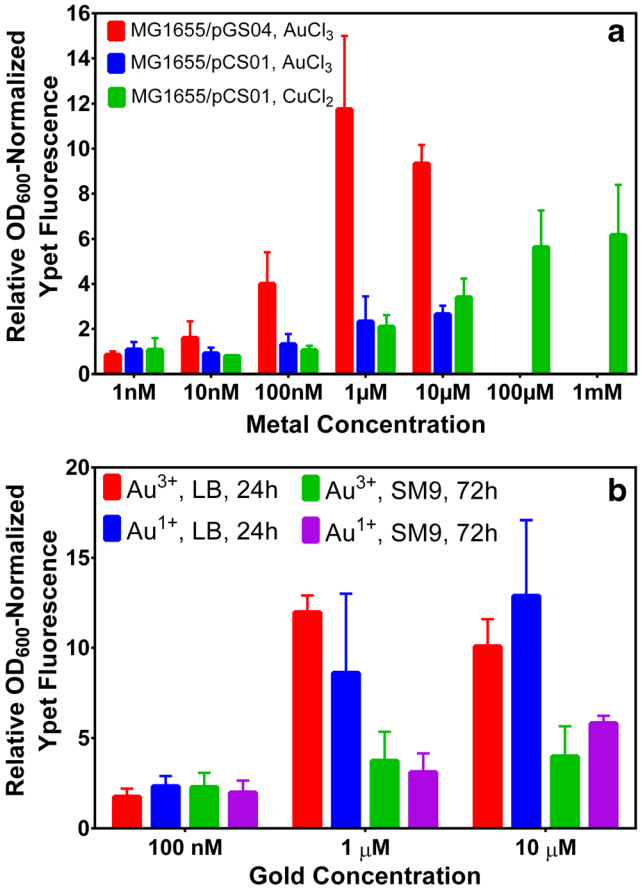

Fig. 5 Abiotic factors affecting gold and copper sensors. **a** Sensitivity characterization of the *E. coli* sensors MG1655/pGS04 and MG1655/pCS01 in SM9 medium to varying concentrations of $AuCl_3$ and $CuCl_2$. The OD_{600}-normalized Ypet fluorescence values for each strain without the addition of metal were used as the reference in Eq. 1. Measurements were taken 24 h after addition of metal to cultures at a starting cell density of $OD_{600} = 0.1$. $AuCl_3$ was not tested at 100 μM and 1 mM due to toxicity. **b** Effect of gold oxidation state on response of the *E. coli* gold sensor MG1655/pGS04 in LB and SM9 media. The OD_{600}-normalized Ypet fluorescence values for MG1655/pGS04 with 10 μM of HCl were used as the reference in Eq. 1. Measurements were taken 24 h after metal addition for cultures in LB medium and 72 h after metal addition for cultures in SM9 medium. Data are averages of at least three biological replicates with error bars indicating the standard deviation

minimal environments, with chemically minimal environments allowing for detection of lower metal concentrations.

The oxidation state of gold was previously shown to effect performance of GolS-based sensors in engineered *E. coli* gold sensors (Zammit et al. 2013). To determine the effect of oxidation state on gold sensing, MG1655/pGS04 was tested with two different gold sources: $AuCl_3$ (Au^{3+}) and $Na_3Au(S_2O_3)_2$ (Au^{1+}). MG1655/pGS04 cultures at a starting OD_{600} of 0.1 were treated with Au^{3+} and Au^{1+} at concentrations of 100 nM, 1 μM, and 10 μM in both complex (LB) and minimal (SM9) media, and the timepoints yielding the maximum responses are shown for each medium (24 h for LB and 72 h for SM9, Fig. 5b). No significant differences were observed in the sensor response when using the Au^{3+} vs Au^{1+} sources at these concentrations and timepoints. The only variations in sensor response to Au^{3+} vs Au^{1+} were transient differences under 10 μM gold at the 8-h timepoint in LB medium and the 48-h timepoint in SM9 medium (Fig. S8, Online Resource 1).

Gold and copper sensors in *Synechococcus* sp. PCC 7002

The gold and copper sensor constructs (pGS03, pGS04, pGS05, and pCS01) were integrated into the *Synechococcus* sp. PCC 7002 genome at putative neutral site 2 to produce cyanobacterial gold and copper sensors (SynGS01, SynGS02, SynGS03, and SynCS01). All fluorescence data for the *E. coli* sensors were collected using a microplate reader; due to the low fluorescence emission from the cyanobacterial sensors, however, the performance of these sensors was evaluated using a flow cytometer to measure Ypet fluorescence emission at the cellular level to minimize re-absorbance of emitted fluorescence by photosynthetic pigments. Only SynGS02 showed a significant response to 1 μM $AuCl_3$ addition, producing a maximum 2.35-fold increase in Ypet fluorescence 2 days after gold addition (Fig. 6a). These results are consistent with the *E. coli* gold sensors in that $GolS^{A113T}$ expression from the *rbc* promoter has the highest fluorescence response to gold addition (Fig. 2b). The GolSCL-based gold and copper sensor, SynCS01, produced a maximum 17% increase in Ypet fluorescence 8 h after the addition of 1 μM $AuCl_3$ but failed to produce a significant response at later timepoints (Fig. 6b). Similarly, the addition of 100 μM of $CuCl_2$ resulted in a maximum 13% increase in Ypet fluorescence 24 h after copper addition to SynCS01 cultures relative to wild-type cultures, but the fluorescence response was indistinguishable from the wild type at later timepoints. While functional gold and copper sensing was demonstrated with the cyanobacterial biosensors, the fluorescence response was significantly lower than that measured with the *E. coli* sensors containing the same constructs.

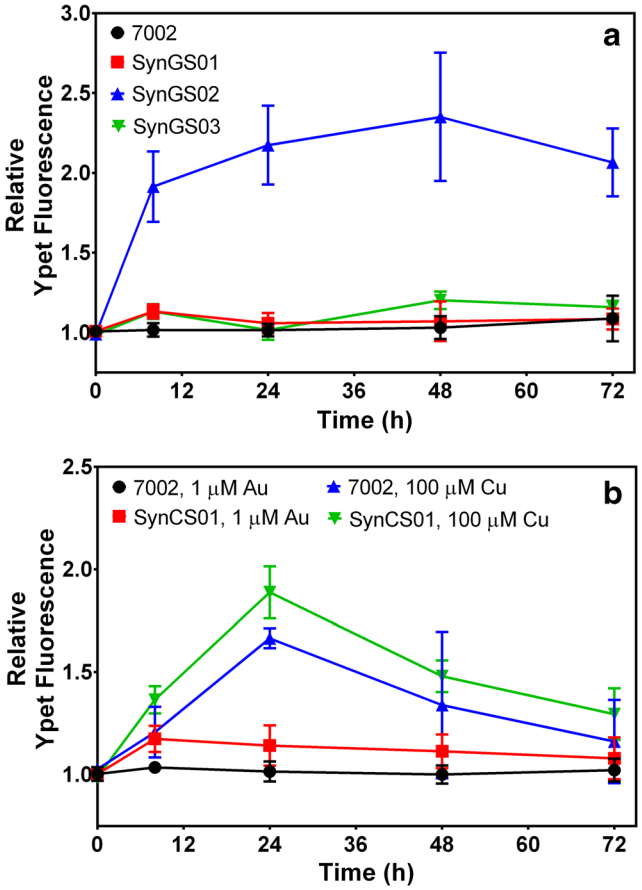

Fig. 6 Characterization of *Synechococcus* sp. PCC 7002 gold and copper sensors. **a** Relative Ypet fluorescence for wild-type *Synechococcus* sp. PCC 7002 and the $GolS^{A113T}$-based gold sensors SynGS01, SynGS02, and SynGS03 with the addition of 1 μM $AuCl_3$ at a starting cell density of $OD_{730} = 0.1$. Mean FL1-A fluorescence values for the cell populations with gold addition are referenced to the same strain without gold. **b** Relative Ypet fluorescence for wild-type *Synechococcus* sp. PCC 7002 and the GolSCL-based gold and copper sensor SynCS01 with 1 μM $AuCl_3$ or 100 μM $CuCl_2$ at a starting cell density of $OD_{730} = 0.1$. Mean FL1-A fluorescence values for the cell populations with metal addition are referenced to the same strain without metal. Data are averages of at least three biological replicates, with error bars indicating the standard deviation

Discussion

This study engineered and optimized gold and copper sensors in a heterotrophic model organism, *E. coli*, and expressed and characterized this system in a marine, photoautotrophic host, *Synechococcus* sp. PCC 7002. The gold sensor system was optimized in *E. coli* by tuning translation of the reporter protein and expression of the gold-activated transcriptional regulator. Addition of a strong RBS from *Synechococcus* sp. PCC 7002 improved translation of the reporter protein, leading to more than a twofold increase in Ypet fluorescence emission from the *E. coli* gold sensor (Fig. 2a). Using the RBS calculator for *E. coli* (Espah Borujeni et al. 2014; Salis et al. 2009), only a 14% increase in the translation rate of *ypet* was

predicted with the addition of the A2579 RBS, yet we measured a 6.9-fold increase in Ypet protein levels (Fig. S2b, Online Resource 1). While Ypet translation improved with the A2579 RBS, this did not improve the signal-to-noise for gold detection. Optimization of *golS*^{A113T} expression improved the fluorescence response to gold in both *E. coli* and *Synechococcus* sp. PCC 7002. In *E. coli*, two methods were used to measure promoter expression: (1) Ypet fluorescence from pSBP$_{1173}$Ypet, pSBP$_{rbc}$Ypet, and pSBP$_{2579}$Ypet and (2) RT-qPCR measurement of *golS*^{A113T} transcripts in MG1655/pGS03, MG1655/pGS04, and MG1655/pGS05. The fluorescence measurements indicated similar promoter strengths for P$_{rbc}$ and P$_{2579}$, yet *golS*^{A113T} transcript levels were 3.2-fold greater in MG1655/pGS04 (containing P$_{rbc}$- *golS*^{A113T}) relative to MG1655/pGS05 (containing P$_{2579}$-*golS*^{A113T}). The *golS*^{A113T} transcript levels correlate with gold sensor performance (Fig. 2b), with increasing *golS*^{A113T} transcript correlating to enhanced Ypet fluorescence response. Similar results were observed in *Synechococcus* sp. PCC 7002, with SynGS02, containing integration of pGS04, showing the greatest Ypet fluorescence response to gold addition (Fig. 6a). However, the A2579 promoter is a stronger promoter relative to the *rbc* promoter in *Synechococcus* sp. PCC 7002 (Ruffing et al. 2016), suggesting that SynGS03 should perform better than SynGS02. SynGS03 displayed reduced chlorophyll-*a* fluorescence with the addition to gold (Fig. S9), suggesting high levels of GolS^{A113T} may negatively affect photosynthesis—possibly through sequestration of copper (Miao et al. 2005). Therefore, optimal performance of the gold sensors in *Synechococcus* sp. PCC 7002 may require proper balancing of the GolS^{A113T} transcriptional regulator.

To compare our gold and copper sensors to previous MerR family metal sensors, we performed extensive characterization of the GolS^{A113T}- and GolSCL-based sensors in *E. coli*, the host most commonly used for metal sensor characterization. The sensitivities of gold and copper detection in the *E. coli* sensors MG1655/pGS04 and MG1655/pCS01 were similar to that reported for other MerR family metal sensors, with a detection limit of 10 nM in minimal medium (Cerminati et al. 2011; Ibanez et al. 2013; Kang et al. 2018; Zammit et al. 2013). The specificity of the GolS^{A113T}-based sensor, MG1655/pGS04, for its target metal (gold) was greater than that of other MerR family sensors which have more promiscuous metal-binding loops. However, the GolSCL-based sensor, MG1655/pCS01, was similar to other CueR and GolS sensors that are activated by both gold and copper (Fig. 4c) (Ibanez et al. 2013, 2015; Stoyanov et al. 2003). Previous studies of CueR indicate that it is activated by silver (Stoyanov et al. 2001), yet in this study, the GolSCL sensor, containing the metal-binding loop from CueR, did not show activation in response to silver (Fig. 4c). Compared to the GolS^{A113T}-based sensor (MG1655/pGS04), the GolSCL-based sensor (MG1655/pCS01) displayed lower levels of Ypet fluorescence in response to metal addition, suggesting that while the CueR metal-binding loop is functional within the GolS protein, it is not optimal for transcriptional activation.

Abiotic factors were also found to effect performance of the metal sensors in *E. coli*. As the chemical matrix of environmental samples may be highly variable, we tested metal sensing performance in both a complex LB medium and a minimal medium (SM9). As expected, the sensitivity of detection improved in the minimal medium, and growth inhibition of the *E. coli* sensors was observed at lower metal concentrations in the minimal medium. These results suggest that gold may be complexing with nitrogen- and sulfur-containing compounds found in the complex LB medium (Checa and Soncini 2011). The oxidation state of gold was previously reported to effect GolS transcriptional activation in an *E. coli* host (Zammit et al. 2013), yet the results in this study do not show a significant change in response to Au^{3+} vs Au^{1+} sources for activation (Fig. 5b). Our results are in agreement with a study on GolS-based fluorescence activation using *Salmonella typhimurium*, which showed similar fluorescence activation with Au^{3+} ($AuHCl_4$) and Au^{1+} ($AuK(CN)_2$) activation sources (Cerminati et al. 2011). It is likely that Au^{3+} is reduced to Au^{1+} intracellularly, and the Au^{1+} form activates GolS^{A113T}, as predicted in previous studies (Checa and Soncini 2011; Ibanez et al. 2013; Stoyanov et al. 2003).

Cyanobacteria, such as the freshwater strain *Synechococcus elongatus* PCC 7942, have previously been engineered to serve as heavy metal sensors for zinc, copper, and cadmium (Erbe et al. 1996), and microalgae are proposed to be ideal sensors for monitoring heavy metals in natural aquatic sources due to their ability to thrive in these ecosystems (Brayner et al. 2011). In this study, we demonstrate that the marine cyanobacterium *Synechococcus* sp. PCC 7002 can be engineered as a gold and copper biosensor. However, the *Synechococcus* sp. PCC 7002 gold sensors exhibited fluorescence emission that was more than three orders of magnitude lower than that measured from the *E. coli* gold sensors, and the GolSCL-based copper sensor produced only a slight temporal response to gold and copper in *Synechococcus* sp. PCC 7002, despite yielding a sixfold increase in Ypet fluorescence in *E. coli* (Figs. 6b and 3). Several factors may contribute to the poor performance of the cyanobacterial sensors relative to the *E. coli* sensors. First, the *E. coli* sensors used plasmid-based expression of the metal sensing construct with a high copy number pUC origin of replication (~300–500 copies) while the cyanobacterial sensors contained genome-integrated sensor constructs with an estimated copy number of ~13 (unpublished data) due to the polyploidy nature of *Synechococcus* sp. PCC 7002 (Griese et al. 2011). The lower copy number of sensor constructs in the *Synechococcus* sp. PCC 7002 host may contribute to the reduced response. Spectral interference from the photosynthetic pigments in

Synechococcus sp. PCC 7002 may also contribute to the lower Ypet fluorescence in the cyanobacterial sensors, as chlorophyll-*a* and the phycobiliproteins will compete with Ypet for excitation light and may also reabsorb fluorescence emitted from Ypet. Despite this potential for spectral interference, our laboratory has measured Ypet fluorescence in modified *Synechococcus* sp. PCC 7002 that is 40-fold greater than the level detected from wild-type *Synechococcus* sp. PCC 7002, suggesting that other mechanisms likely contribute to the low fluorescence response of the cyanobacterial gold sensors (Ruffing et al. 2016). Another reason for low activation in *Synechococcus* sp. PCC 7002 may be the structural differences in RNA polymerase (RNAP) between the natural host *S. enterica* and *Synechococcus* sp. PCC 7002. Cyanobacterial RNAPs have been shown to contain a split β′ subunit (RpoC1 and RpoC2) relative to the single β′ subunit (RpoC) found in other eubacteria such as *E. coli* and *S. enterica* (Bergsland and Haselkorn 1991; Schneider and Hasekorn 1988; Xie et al. 1989). Structural differences in the RNAP of *Synechococcus* sp. PCC 7002 relative to *S. enterica* may affect the ability of the RNAP to interact with the *golB* promoter when activated by gold binding to the transcriptional activator $GolS^{A113T}$. Lastly, differences in gold and copper uptake may result in lower responses of the cyanobacterial sensors relative to the *E. coli* sensors. Copper homeostasis in cyanobacteria has previously been explored, but less is known about gold homeostasis (López-Maury et al. 2012). If metal uptake in *Synechococcus* sp. PCC 7002 is insufficient to support activation of the gold and copper sensors, increasing the metal concentrations would be expected to improve the response of the sensor due to enhanced diffusion across the membrane. We found that increasing the $AuCl_3$ concentration to 10 μM resulted in growth inhibition of the cyanobacterial sensors (data not shown), suggesting that gold uptake is sufficient. Future engineering efforts may improve the performance of the cyanobacterial gold and copper sensors, such as increasing the copy number of the sensor construct, expression of a RNAP that is compatible with the *golB* promoter, or mutation of the *golB* promoter. Additionally, the use of other copper-regulated promoters that have been previously studied in cyanobacteria may serve as potential alternatives for the development of copper biosensors (Huang and Lindblad 2013; López-Maury et al. 2012). Despite the low response of the cyanobacterial gold sensors, this study provides a proof-of-concept demonstration that cyanobacterial hosts can be modified as whole cell biosensors for heavy metals in aquatic environments.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical statement This article does not contain any studies with human participants or animals performed by any of the authors.

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